



BIOSAFETY CONSIDERATIONS FOR ANIMAL EXPERIMENTS

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Biosafety/Biosecurity

- More prominent and important than ever
- Lots of attention being paid now
- Are we ready?
- New facilities for research and diagnosis being build to meet the challenge



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Laboratory accidents

NEWS OF THE WEEK

BIOSECURITY

Reports Blame Animal Health Lab In Foot-and-Mouth Whodunit

Neglected, leaky pipes and England's record-setting wet summer likely combined to cause the country's recent outbreak of foot-and-mouth disease (FMD), according to two reports issued last week. The virus responsible probably escaped from a company, Merial, that grew vast amounts of it for vaccine production, the studies say. Yet the reports assign most of the blame for the outbreak to the Institute for Animal Health (IAH), a government lab at the same site in Pirbright that owned the aging network of underground wastewater pipes and was aware that it needed maintenance. IAH breached biosecurity in

IAH and also funds some 65% of its work.

Genomic comparisons of the outbreak virus to strains from Merial and IAH can't pinpoint from which of the two labs the virus escaped, according to the reports, one led by the U.K.'s Health and Safety Executive (HSE), a government agency, and the other by molecular epidemiologist Brian Spratt of Imperial College London. Still, the panels say, it's much more likely that the virus came from Merial, which grew it in two 6000-liter vats shortly before the accident, producing a million times more virus than IAH used in its small-scale experiments.

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Laboratory breach

NEWS OF THE WEEK



BIODEFENSE RESEARCH

Lapses in Biosafety Spark Concern

An apparent breakdown in biosafety at Texas A&M University (TAMU) in College Station is prompting scrutiny of the expansive

was no evidence that a coordinated response or biosafety assessment was performed as a result of these exposures,

Under fire, Texas A&M University was faulted for lapses in its oversight of pathogen research.

dures. Many of the TAMU violations, however, concern access to pathogens and lab practices.

The problems CDC cited are serious but probably not unique, according to scientists both inside and outside TAMU. "If you were to apply an equivalent level of scrutiny at other institutions, I think you would find issues of concern," says TAMU microbiologist Vernon Tesh, one of four lab leaders singled out for safety lapses in CDC's report. "You always have to have safety in mind," he added. "Having said that, accidents happen." In a press conference last week, TAMU's interim president Eddie Davis said that other "institutions under that same level of review would probably have findings that would be reportable to the CDC."

Since the CDC's July inspection, the university's vice president of research and overseer of biosafety compliance, Richard Ewing, has resigned from his position and returned to the mathematics department (*Science*,

g on September 24, 2007

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BIOSAFETY BREACHES

Accidents Spur a Closer Look at Risks at Biodefense Labs

Failure to report a *Brucella* infection and other problems at a Texas university have microbiologists searching for ways to ensure safety and public trust

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Administrative Considerations

The key organizations in the review of animal/biohazard experiments:

- Institutional Animal Care and Use Committee(IACUC)
- Institutional Biosafety Committee (IBC)
- Environmental Health and Safety/ Biosafety Officer
- Attending Veterinarian

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The Risk Assessment

- Virulence/pathogenicity/infectious dose
- Environmental stability of the agent
- Route of spread, communicability
- Quantity/concentration/volume of agent used
- Vaccine/Treatment availability
- Allergenicity.

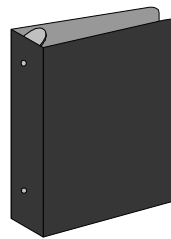
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Classification of Agents

International bodies (WHO, CDC, CHA) have classified agents into Risk groups on a scale from 1 through 4.

1 = Mild

4 = Severe



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Risk Assessment

- Routes of Transmission of the Agent
- Medical Surveillance
- Engineering Controls
- Personal Protective Equipment Use
- Facility Design, both laboratory and vivaria
- Zoonoses
- Vectors

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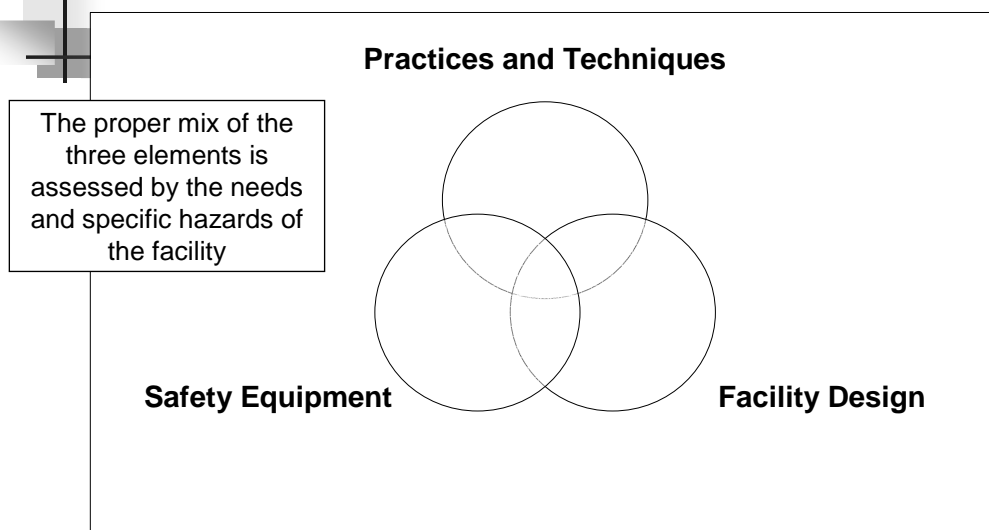
Biosafety

- What is **biosafety** for animal experiments?
The application of combinations of laboratory practice and procedure, vivarium facilities, and safety equipment when working with potentially infectious microorganisms.

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Principles of Laboratory Biosafety



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Biosafety Hazards when Working with Laboratory Animals

- **Sharps:** claws, teeth, surgical instruments, edges on animal cages
- **Zoonoses:** human pathogens which are often asymptomatic in the animal
- **Surface Contact:** blood, body fluids
- **Allergens:** dander, fur, dust, bedding
- **Vectors:** insects, parasites

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Routes of Exposure in Animal Procedures

- Parenteral Inoculation (*needle stick*)
- Subcutaneous wound (*bite or scratch*)
- Surface Contact (*contaminated work area*)
- Ingestion (*hand to mouth, food in lab*)
- Inhalation (*aerosol-generating procedures*)
- Ocular (*hand to eye, cleaning infected bedding*)
- Mucous Membrane (*aerosol droplets in face*)

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Good Work Practice

No Eating, Drinking, or Smoking

Ensure that workers are aware of ingestion hazards when working with biohazards and animals:

- Eating, drinking, and smoking are not permitted in animal work areas.
- Hands must be washed with disinfectant hand soap prior to leaving the room.

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Good Work Practice

Limit Access to Animal and Lab Facilities

Ensure that the PI and Animal care personnel limit access to animal biohazard containment areas:

- Access to animal areas shall be **limited to essential personnel** only.
- Visitors may not know the rules of safety in lab areas and may also bring **contamination from the outside** which may affect the health of the animals.

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Good Work Practice

Label All Animal Biohazard Areas

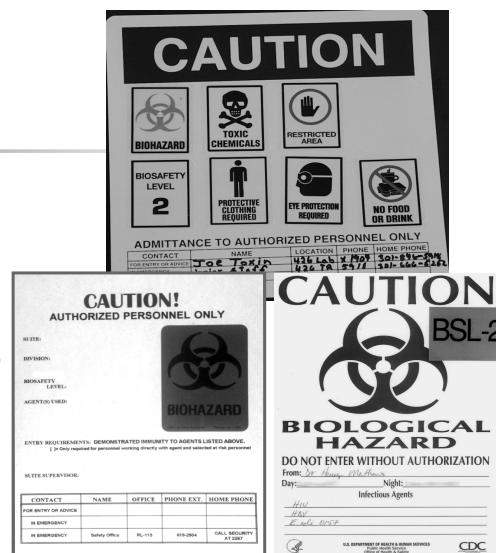
PI posts appropriate biohazard signs with the universal biohazard symbol on the entrance to biohazard use areas to warn the public that biohazardous materials are being used within the room. All PI's must:

- *Label all contaminated equipment in the room (incubator, freezer) with a biohazard label.*
- *Dispose of pathological waste in a red bag labeled with the biohazard symbol.*

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Signage

- **Signage is important to communicate the potential hazard**
- **Post a biohazard warning sign when immunizations or use of respirators is needed for entry**
- **Provide contact information**
- **Persons at increased risk of infection should not enter infected animal rooms**



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Good Work Practice

Hand washing



Ensure hand washing procedures are followed

Hands should be washed:

- with warm water and a disinfectant hand soap.
- immediately and thoroughly if contaminated with blood or other body fluids.
- immediately after gloves are removed.

A foot and elbow operated sink is required for Animal Biosafety Level 3 work.

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Contact With Animal Blood

Review procedures that may involve release of blood or body fluids, such as necropsy:

- The body fluids of infected animals may be a potential source of disease.
- Wild or feral animals may not be screened for all potential diseases and many organisms are asymptomatic in animals.

! *Therefore, handle animal blood as if it were infectious, similar to the approach taken with human specimens (Universal Precautions).*

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Safety Equipment



- Protective clothing with a solid front may be worn in the vivarium.
- Eye protection as needed
- Respiratory protection as dictated by the risk assessment
- Head covering as needed
- Clothing is changed and when contaminated.

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Safety Equipment

Vivarium workers should wear two pairs of gloves when appropriate.



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Good Work Practice

Decontaminate Work Areas

Verify that the disinfectant used will be effective for the agent used. Many disinfectants do not kill a broad spectrum of organisms. Animal laboratory work surfaces should be decontaminated:

- after a spill of blood or biohazardous materials
- when work activities are complete
- with an appropriate chemical disinfectant

! *If animals are left in the room, be sure that fumes from the disinfectant will not harm the animals.*

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Decon Red Bag Waste



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Good Work Practice

Minimize Inhalation Risks

Review inhalation risks to animal handlers when personnel are:

- using high pressure hoses to clean surfaces
- cleaning animal bedding and cages
- handling the animals themselves

In addition, animals shed fur and hair which may exacerbate allergies. For this reason, animal care personnel should wear respiratory protection.

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Allergens

Assess the following risks:

- Animals shed **hair and fur** which may exacerbate existing allergies.
- Animal **bedding** may also be a potential source of allergens and must be handled in a manner to minimize aerosols.
- Animal **urine** may have proteins that cause allergies in humans.
- Animals may also track **dirt or dust** from outside that can cause discomfort.

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Good Work Practice

Use Caution When Cleaning Bedding

Be aware of the hazards of infected bedding, especially those instances where bedding may pose a respiratory hazard.

Example: *Coxiella burnetii*

- disease carried by sheep
- easily airborne
- may infect workers without respiratory protection

Protection: **Workers should wear masks before carefully removing bedding** from areas which may be infected with highly respirable organisms.

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Cage change stations



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Respiratory Safety Equipment

- A Powered Air-Purifying Respirator (PAPR) can also be worn
- Consists of:
 - Disposable hood
 - Breathing tube
 - Motor/blower unit
 - Cartridges
 - Rechargeable battery pack



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Animal in BSC



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Proper Disposal of Animal Carcasses

The PI must first place the carcass in a red bag avoid disposal in the regular trash. However, final disposal will depend of the carcass was also contaminated with hazardous chemicals or radioactive materials.

- **ANIMAL CARCASSES MUST NOT DISPOSED OF IN THE REGULAR TRASH!**

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Good Work Practice Minimize Sharps Use

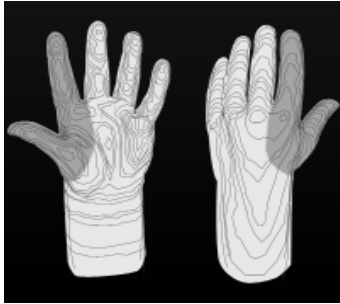
Review all sharps usage:

- Sharps must be handled with extreme caution. Unless animals are restrained, they may squirm and result in accidental self-inoculation (gas anesthesia).
- Needles must not be recapped, bent, sheared, or removed from a disposable syringe.
- All used sharps must be placed in a rigid, hard-plastic, puncture-resistant container for disposal.

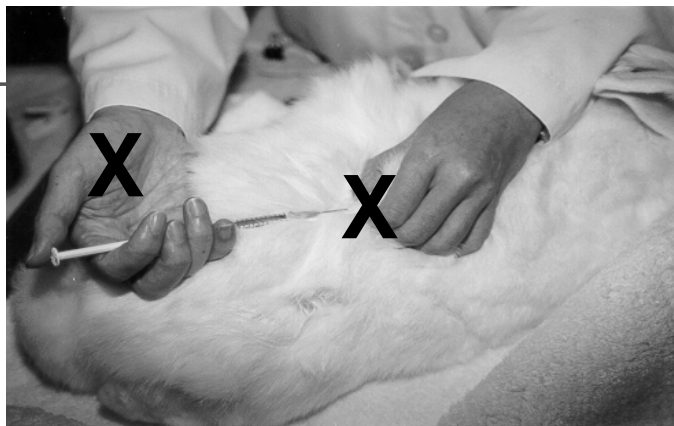


LLC

Finger Armour

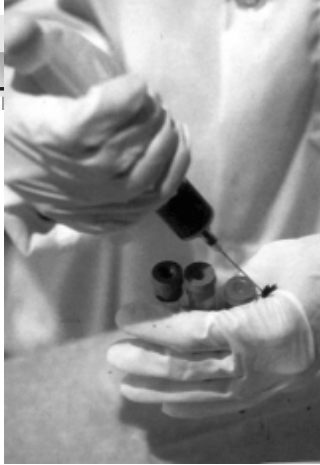


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DO NOT !!!



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Restraint Devices

Animal Grabber



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Rabbit Restraint



Mouse Restraint



Snuggle Restraint



Snake Bag



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Animal Biosafety Levels

There are four laboratory and four (+) animal biosafety levels which consist of combinations of:

- work practices
- safety equipment
- facilities

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Animal Biosafety Level 1

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving **little or no known potential hazard** to animal handling personnel and the environment.

- Work is conducted in the open (bench or cage).
- Special containment equipment, such as a biosafety cabinet, is generally not required.
- *E. coli* K-12 is an example of a organism typically used at ABSL-1.

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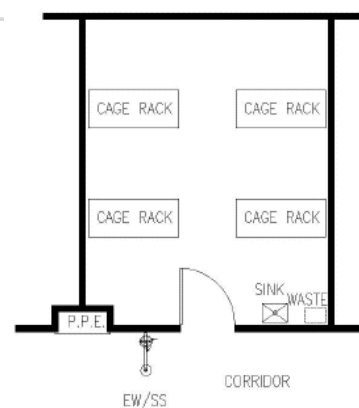
Animal Biosafety Level 1

- Lab coats or gowns are worn in the animal facility.
- Bedding materials are removed in such a manner to minimize aerosol generation.
- Cages are cleaned and disinfected on a regular basis.

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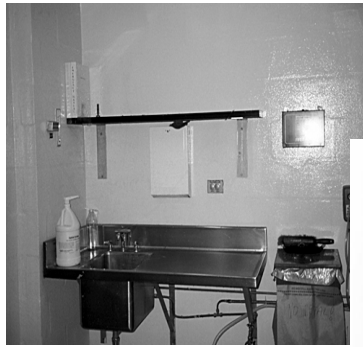
ABSL-1

- Facility is separated from main traffic
- Restricted access
- Doors – self closing and self locking, kept closed, open inward
- Hand wash sink
- No air recirculation
- *Recommended* negative pressure



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ABSL-1 Animal Room



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Animal Biosafety Level 2

Animal Biosafety Level 2 (ABSL-2) is similar to ABSL-1, but work at this level **requires containment devices such as biosafety cabinets whenever aerosol generating procedures are conducted.**

- Specialized training required.
- Access to the facility is limited while the experiment is in progress
- An example of an organism requiring ABSL-2 is *Salmonella*

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Animal Biosafety Level 2

- A biohazard sign is posted on the outside door.
- All personnel must receive appropriate training on the hazards associated with the research.
- All biohazardous agents used in the animal facility must be transported in a manner to minimize leakage. Agents must be transported using double containment in a labeled container.

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Animal Biosafety Level 2

- Medical surveillance appropriate to the animal species used shall be offered to workers prior to the initiation of the experiment.
- Protective clothing worn in the animal use areas shall not be worn outside the room.
- Work areas are disinfected at the conclusion of the experiment.
- Autoclave nearby for trash, bedding and cages

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Animal Biosafety Level 2

Facility Considerations

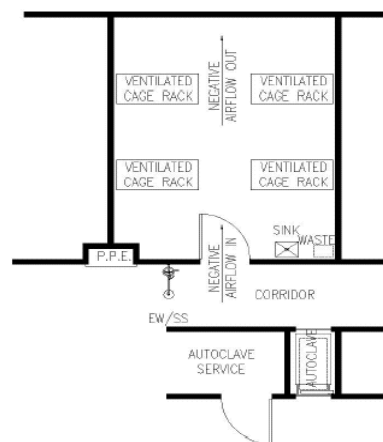
Check for the following safety equipment:

- A **sink** should be available for hand-washing.
- An **autoclave** should be available to treat infected animal bedding prior to disposal.
- A **biosafety cabinet** should be available for aerosol-generating procedures, such as vortexing animal specimens.

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Animal Biosafety Level 2

- Consider room traffic flows
 - Single entry vs. exit
 - Clean/dirty double door concept
 - Clothes change
 - Autoclave nearby

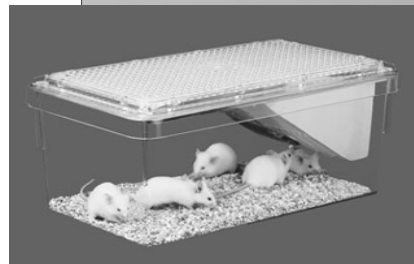
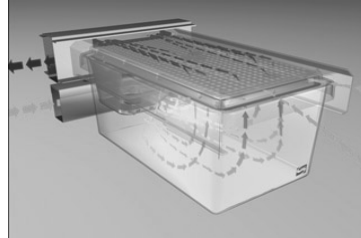


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Animal Caging Systems

When needed, partial or full containment caging systems as per risk assessment

Eg: Rodent-filtered top caging, HEPA-filtered primary barrier systems appropriate to species



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Animal Biosafety Level 3

Animal Biosafety Level 3 (ABSL-3) differs from ABSL-2 in that the **agents may cause serious or lethal disease in animals or man.**

- All ABSL-3 procedures are conducted within a biosafety cabinet and the workers may also be required to wear personal protective equipment such as a respirator.
- The lab has special engineering features to prevent a release of the agent to the environment.
- An example of an agent requiring ABSL-3 containment is *Mycobacterium tuberculosis*

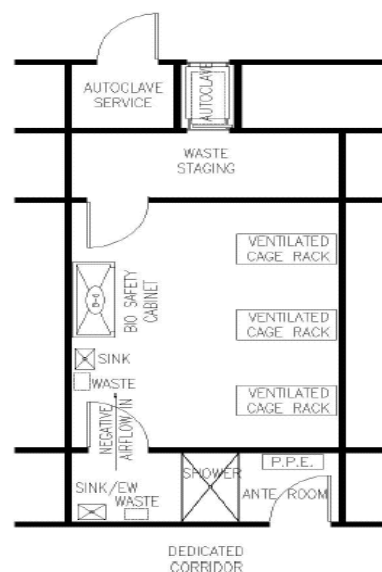
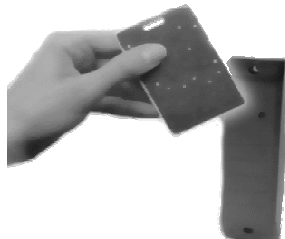
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Animal Biosafety Level 3

- All wastes from the ABSL-3 animal room are **autoclaved** before disposal.
 - All pathological waste from the animal room shall be transported in a leak-proof container before disposal and eventual incineration.
- **Appropriate face and respiratory protection** is worn by personnel entering the animal housing areas.
- All experimental procedures shall be performed in such a manner so as to minimize aerosol generation.
- **NO** sharps

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ABSL 3



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Ventilated Caging Systems



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Animal Biosafety Level 4

Animal Biosafety Level 4 (ABSL-4) is required for all work with dangerous and exotic agents which pose a **high individual risk of infection to animals and man which can be life threatening.**

Animal Biosafety Level 3 (Ag) is like ABSL-4 in facility design and containment, but does not necessarily protect the worker

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Animal Biosafety Level 4

The following are examples of agents used at Animal Biosafety Level 4:

- *Ebola*
- *Marburg*
- *Lassa Fever Virus*
- *Machupo*

Only a very few facilities may accommodate ABSL-4 experiments.

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Medical Surveillance Considerations Vaccinations

The PI should be aware of the availability of vaccines for certain organisms.

- OSHA requires that Hepatitis B virus (HBV) vaccinations must be offered to at risk personnel.
- Other vaccinations, such as rabies and yellow fever are licensed and clearly have benefit for the user.
- Some vaccinations, however, are experimental and are may not be available for general use.

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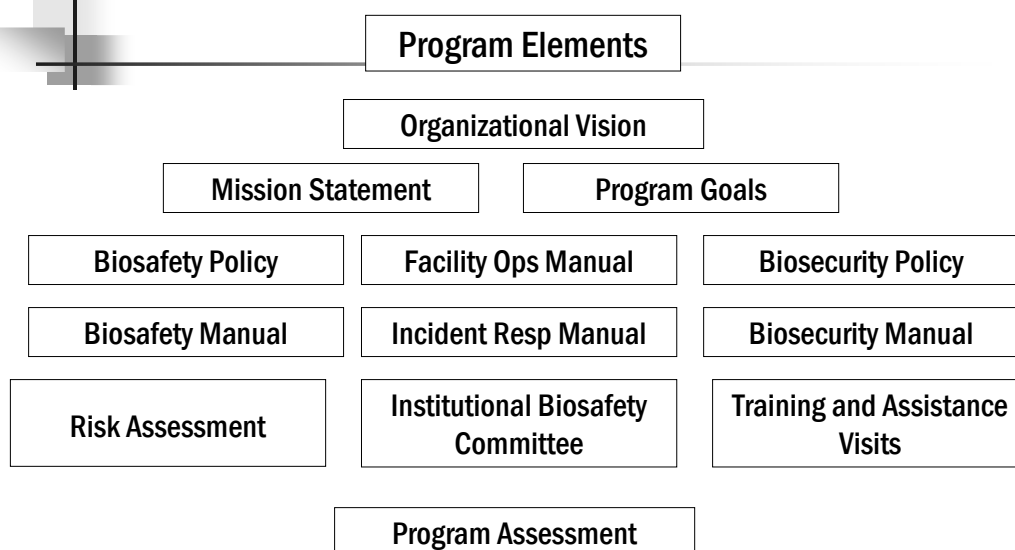
Emergency Procedures

The IBC and IACUC must review emergency procedures for personnel who are bitten or scratched while handling infected animals.

- Animal handlers must report the incident to their supervisor and then to a qualified physician.
- The physician preferably would be familiar with animal zoonoses and other health complications associated with the handling of animals.
- The animal worker may have to provide a specimen in conjunction with post exposure follow up, such as with Herpes B Virus surveillance.

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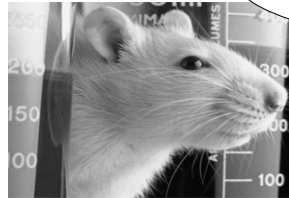
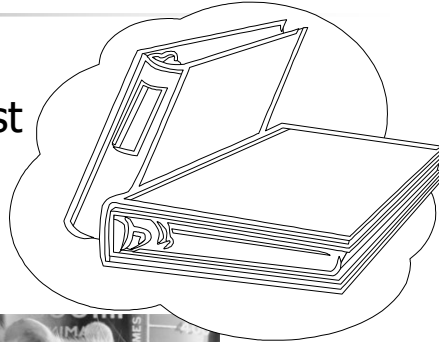
Bio-Risk Management Program



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MANUALS and SOPs

- A safety/security and operations manual must be prepared or adopted.
- Emergency response protocols
- SOPs



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Conclusion

It is essential that all animal biosafety operations are screened for biohazard usage and zoonotic risks. Using laboratory animals is a potential risk in addition to the handling of biohazardous agents. Animals bite and scratch and housekeeping for animals also may require additional efforts. Once the animal and agent use procedures are identified, then appropriate risk management can commence.

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Is it possible?



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THANK YOU

- Robert J. Hashimoto
- Shanna Nesby
- Joe Kozlovac

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